Appendix H

Support for Claims 202, 203 and 206 in Grandparent ('691) and Great-Grandparent ('440) Applications

Disclosures:

Serial No.	Filing Date	Application family
09/265,**191**	3/10/99	CON of 08/593,554
08/593,**554**	1/30/96	CIP of 08/446,691
08/446,**691**	6/7/95	CIP of 08/112,440
08/112,**440**	8/26/93	

Claim #	Claim Limitation	Support in Applicants' Disclosure
202.	A composition comprising: a plasmid including an immunostimulatory nucleic acid sequence...	*'691:* p. 51, line 1, to p. 53, line 7: Examples II, III; p. 66, line 16, to p. 67, line 21: Examples X, XI; p. 68, line 11, to p. 75, line 7: Examples XIII-XVIII: Examples include administration of expression vectors encoding influenza ribonucleoprotein (RNP or NP), ovalbumin (OVA) or βgal. Administration of expression vectors encoding antigens resulted in induction of both NP (antigen)-specific CTLs and anti-NP antibodies (Examples II, III, X, XI, XIV) and OVA-specific CTLs but not anti-OVA antibodies (Example XV). Vaccination of mice with an expression vector (pCMVRNP) protected animals from a lethal challenge of influenza virus (Example XIII). Administration of an expression vector encoding lacZ resulted in generation of anti-βgal antibodies, in particular, Th1-type antibodies (IgG2a) whereas administration of βgal protein resulted in Th2-type antibodies (IgG1) (Example XVI, XVII, XVIII). *'440:* p. 35, line 1, to p. 37, line 7: Examples II, III; p.50, line 16, to p. 51, line 19: Examples X, XI; p. 52, line 16, to p. 53, line 19: Examples XIII, XIV: Examples include administration of expression vectors encoding influenza ribonucleoprotein (RNP) or βgal. Administration of expression vectors encoding antigen resulted in induction of both antigen-specific CTL and anti-antigen antibodies (Examples II, III, X, XI and XIV). Vaccination of mice with an expression vector (pCMVRNP) protected animals from a lethal challenge of influenza virus (Example XIII).

Claim #	Claim Limitation	Support in Applicants' Disclosure
	...comprising AACGTT, wherein C is unmethylated, ...	*'691:* p. 33, lines 1-2: "Other preferred plasmid vectors are pREP7 and pREV which are commercially available from Invitrogen of San Diego, California." *'691:* p. 32, lines 18-19: "Suitable plasmid vectors are well-known in the art and include the vectors described in *Current Protocols in Molecular Biology*, supra at Ch. 1." *'440:* p. 23, lines 17-18: "Other preferred plasmid vectors are pREP7 and pREV which are commercially available from Invitrogen of San Diego, California." *'440:* p. 23, lines 8-9: "Suitable plasmid vectors are well-known in the art and include the vectors described in *Current Protocols in Molecular Biology*, supra at Ch. 1." Since grown in bacteria, the vector DNA is unmethylated. The pREP7 plasmid contains immunostimulatory CG formulas (*e.g.*, two AACGTT in the Amp gene). Please see Appendix I for map of pREP7 and GenBank accession number J01749 (sequence for pBR322 which contains the ampicillin resistance gene (Amp)).

Claim #	Claim Limitation	Support in Applicants' Disclosure
	...and an antigen in a pharmaceutically acceptable carrier, wherein the antigen is encoded in the plasmid.	*'691:* p. 28, lines 5-8: "For example, the naked polynucleotides may operatively encode for therapeutic peptides, but will preferably encode for immunogenic peptides which can act as antigens to provoke a humoral and/or cellular response." *'691:* p. 32, lines 11-12: "A particularly preferred form of a naked polynucleotide for use in the invention will be one which has been incorporated into a plasmid vector." *'691:* p. 51, line 1, to p. 53, line 7: Examples II, III; p. 66, line 16, to p. 67, line 21: Examples X, XI; p. 69, lines 1-15: Example XIV; p. 71, line 1, to p. 75, line 7: Examples XVI-XVIII: Examples include administration of expression vectors encoding influenza ribonucleoprotein (RNP or NP), ovalbumin (OVA) or βgal. Administration of expression vectors encoding antigens resulted in induction of both antigen-specific CTLs and anti-antigen antibodies (Examples II, III, X, XI, XIV). Administration of an expression vector encoding lacZ resulted in generation of anti-βgal antibodies, in particular, Th1-type antibodies (IgG2a) whereas administration of βgal protein resulted in Th2-type antibodies (IgG1) (Example XVI, XVII, XVIII). *'691:* p. 36, lines 25-26: "Compositions of naked polynucleotides and mixtures of polynucleotides may be placed into a pharmaceutically acceptable suspension, solution or emulsion."

Claim #	Claim Limitation	Support in Applicants' Disclosure
		'440: p. 22, lines 5-7: "The naked nucleotides may operatively encode for therapeutic peptides, but will preferably encode for immunogenic peptides which can act as antigens to provoke a humoral and/or cellular response." *'440:* p. 23, lines 1-2: "A particularly preferred form of a naked nucleotide for use in the invention will be one which has been incorporated into a plasmid vector." *'440:* p. 35, line 1, to p. 37, line 7: Examples II, III; p.50, line 16, to p. 51, line 19: Examples X, XI; p. 53, lines 6-19: Example XIV: Examples include administration of expression vectors encoding influenza ribonucleoprotein (RNP) or βgal. Administration of expression vectors encoding antigen resulted in induction of both antigen-specific CTL and anti-antigen antibodies (Examples II, III, X, XI and XIV). *'440:* p. 24, lines 15-16: "Compositions of naked nucleotides and mixtures of nucleotides may be placed into a pharmaceutically acceptable suspension, solution or emulsion."

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203.	The composition of claim 202, wherein the plasmid is pREP7 encoding an antigen.	*'691:* p. 33, lines 1-2: "Other preferred plasmid vectors are pREP7 and pREV which are commercially available from Invitrogen of San Diego, California." *'691:* p. 48, lines 7-9: "A 1040 bp HindIII-XhoI fragment containing the V-J region of [the kappa light chain] gene was excised and inserted into the polycloning site of the mammalian expression vector pREP7 (Invitrogen, San Diego, CA).....to produce a vector designated pREVk3." *'440:* p. 23, lines 17-18: "Other preferred plasmid vectors are pREP7 and pREV which are commercially available from Invitrogen of San Diego, California." *'440:* p. 32, line 22, to page 33, line 1: "A 1040 bp HindIII-XhoI fragment containing the V-J region of [the kappa light chain] gene was excised and inserted into the polycloning site of the mammalian expression vector pREP7 (Invitrogen, San Diego, CA).....to produce a vector designated pREVk3."

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206.	A pharmaceutical composition for stimulating an immune response to an antigen,...	*'691:* p. 51, line 1, to p. 53, line 7: Examples II, III; p. 66, line 16, to p. 67, line 21: Examples X, XI; p. 68, line 11, to p. 75, line 7: Examples XIII-XVIII: Examples include administration of expression vectors encoding influenza ribonucleoprotein (RNP or NP), ovalbumin (OVA) or βgal. Administration of expression vectors encoding antigens resulted in induction of both NP (antigen)-specific CTLs and anti-NP antibodies (Examples II, III, X, XI, XIV) and OVA-specific CTLs but not anti-OVA antibodies (Example XV). Vaccination of mice with an expression vector (pCMVRNP) protected animals from a lethal challenge of influenza virus (Example XIII). Administration of an expression vector encoding lacZ resulted in generation of anti-βgal antibodies, in particular, Th1-type antibodies (IgG2a) whereas administration of βgal protein resulted in Th2-type antibodies (IgG1) (Example XVI, XVII, XVIII). *'691:* p. 36, lines 25-26: "Compositions of naked polynucleotides and mixtures of polynucleotides may be placed into a pharmaceutically acceptable suspension, solution or emulsion." *'440:* p. 35, line 1, to p. 37, line 7: Examples II, III; p.50, line 16, to p. 51, line 19: Examples X, XI; p. 52, line 16, to p. 53, line 19: Examples XIII, XIV: Examples include administration of expression vectors encoding influenza ribonucleoprotein (RNP) or βgal. Administration of expression vectors encoding antigen resulted in induction of both antigen-specific CTL and anti-antigen antibodies (Examples II, III, X, XI and XIV). Vaccination of mice with an expression vector (pCMVRNP) protected animals from a lethal challenge of influenza virus (Example XIII). *'440:* p. 24, lines 15-16: "Compositions of naked nucleotides and mixtures of nucleotides may be placed into a pharmaceutically acceptable suspension, solution or emulsion."

Claim #	Claim Limitation	Support in Applicants' Disclosure
	...comprising pREP7 encoding the antigen and a pharmaceutically acceptable carrier.	*'691:* p. 33, lines 1-2: "Other preferred plasmid vectors are pREP7 and pREV which are commercially available from Invitrogen of San Diego, California." *'691:* p. 48, lines 7-9: "A 1040 bp HindIII-XhoI fragment containing the V-J region of [the kappa light chain] gene was excised and inserted into the polycloning site of the mammalian expression vector pREP7 (Invitrogen, San Diego, CA).....to produce a vector designated pREVk3." *'691:* p. 36, lines 25-26: "Compositions of naked polynucleotides and mixtures of polynucleotides may be placed into a pharmaceutically acceptable suspension, solution or emulsion." *'440:* p. 23, lines 17-18: "Other preferred plasmid vectors are pREP7 and pREV which are commercially available from Invitrogen of San Diego, California." *'440:* p. 32, line 22, to page 33, line 1: "A 1040 bp HindIII-XhoI fragment containing the V-J region of [the kappa light chain] gene was excised and inserted into the polycloning site of the mammalian expression vector pREP7 (Invitrogen, San Diego, CA).....to produce a vector designated pREVk3." *'440:* p. 24, lines 15-16: "Compositions of naked nucleotides and mixtures of nucleotides may be placed into a pharmaceutically acceptable suspension, solution or emulsion."